SCIENTIFIC NOTE

Mosquito Species Breeding in Bromeliad Axils on the Island of Kauai, Hawaii

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Abstract. Bromeliads are important ornamental plants in Hawaii. They grow widely in yards, gardens and commercial nurseries. The water held in bromeliads provides breeding sites for mosquito larvae. A survey was conducted from May to September 2001 at four sites on the island of Kauai, Hawaii. Choice of three species of bromeliads for the survey was based solely on the volume of reservoirs of water held in their axils. They were *Vriesea aff. regina*, *Neoregelia* sp. and *Neoregelia* '*Macwilliamsii*'. Four species of mosquitoes were recovered, including a bromeliad mosquito, *Wyeomyia mitchellii* (Theobald), the Asian tiger mosquito, *Aedes albopictus* (Skuse), the southern house mosquito, *Culex quinquefasciatus* (Say), and a beneficial species, *Toxorhynchites amboinensis* (Doleschall). The abundance of mosquito larvae was affected by bromeliad species. The larvae of *Wy.* m*itchellii*, *Ae. albopictus* and *T. amboinensis* were found in all three species of bromeliads while *Cu. quinquefasciatus* was only recovered from *V. aff. regina.* Overall, *V. aff. regina*, the largest of the three species, contained more mosquito larvae, both in numbers and species, than the other two species of bromeliads. The abundance of mosquito larvae in bromeliads was also affected by their location. For the three noxious species of mosquitoes, the number of larvae present in the axils of the three species of bromeliads varied from site to site. For *V. aff. regina,* more *Cu. quinquefasciatus* larvae were found in the leaf axils than in the central axils. The three noxious species of mosquitoes can either breed alone or together in *V. aff. regina* axils.

Key words: bromeliad, *Aedes albopictus* , *Wyeomyia mitchellii*, *Culex quinquefasciatus*

Introduction

Bromeliads (Bromeliaceae) are the foremost plants used for landscaping, cut flowers and live floral arrangements in the world (Parkhurst 2000). However, the water held in bromeliads provides breeding sites for many different species of mosquitoes (Frank and Curtis 1981).

The bromeliad mosquito, *Wyeomyia mitchellii* (Theobald), was discovered on Oahu in 1981 (Shroyer 1981), and has spread to Kauai and Hawaii (D. Jamieson, personal communication), but is thus far absent from Maui, Lanai and Molokai. The Asian tiger mosquito, *Aedes albopictus* (Skuse), was introduced into Hawaii in the 1890s (Joyce 1961). It is an important vector of dengue and other human viruses and capable of transmitting a wide range of viruses, including yellow fever, Japanese and St. Louis encephalitis, and several California group Bunyaviruses (Hawley et al. 1987). *Aedes albopictus* was responsible for the outbreak of dengue in summer 2001 in Hawaii. The southern house mosquito, *Culex quinquefasciatus* (Say), was introduced into Hawaii in 1868 (Joyce 1961). It is not only the vector of filariasis and West Nile virus of humans, but also the vector of bird malaria, which is blamed for the disappearance of many native birds from lower elevations in Hawaii. *Toxorhynchites amboinensis* (Doleschall), a beneficial species of mosquito, was introduced into Hawaii as a biological control agent of *Ae. albopictus* in 1953 (Steffan 1968). It is established on Kauai, and often found in artificial containers, in which *Ae. albopictus*s breeds.

Because of their medical importance, *Ae. albopictus,* and *Cu. quinquefasciatus* have been well studied from different aspects. However, little is known of their breeding behavior in bromeliads. Very little information has been published on *Wy. mitchellii* due to its restricted breeding habitats and unknown vector status. Although *T. amboinensis* was introduced to control *Ae. albopictus,* very little is known of its breeding habits in bromeliad axils.

On Kauai, bromeliads are widely grown in back yards, gardens, and commercial nurseries. In our pre-survey, we recovered all three noxious species of mosquitoes from bromeliad axils (Furumizo and Yang unpublished data). In the present study, we investigated the larval abundance and distribution of these three species and *Toxorhynchites amboinensis* in bromeliad plants.

Materials and Methods

Four separate locations were chosen as survey sites, which included (1) the Plantation Garden at Poipu, (2) the National Tropical Botanical Garden at Lawai, (3) a nursery at Kilauea and (4) another nursery at Princeville. All sites are located along the coastal areas with an annual rainfall between 101 and 152 cm. All of the bromeliads held water due to rainfall and watering. Three species of bromeliads were chosen for the survey based on their size and the volume of water in their axils. They were *Vriesea aff. regina*, *Neoregelia* sp., and *Neoregelia* '*Macwilliamsii*' (Parkhurst 2000; Rauch and Weissich 2000). *Vriesea aff. regina* and *Neoregelia* sp. grow as individual plants and *N.* '*Macwilliamsii*' grows in large mats. All three species possess a water-filled central cup (central axil) and only *V. aff. regina* has numerous large surrounding water-filled leaf axils. We measured the volumetric capacity of the axils by filling each axil with water to its maximum holding capacity as described by Frank et al. (1977). Ten central axils of each species were measured except for *V. aff. regina,* for which 10 more leaf axils were measured.

Unlike *V. aff. regina,* one or the other of the additional two species of bromeliads could not be found in sufficient numbers at the Kilauea or Princeville nurseries. Therefore, only two species were sampled at these sites (Table 1).

From May to September 2001, each bromeliad species at each site were randomly sampled at monthly intervals. For *V. aff. regina,* samples were taken from the central axils and from the leaf axils. However, for the other two species, samples were taken only from the central axils. The total number of axils sampled from each species at each site is shown in Table 1. The water of each sample was siphoned using an Aquatic pipette (BioQuip Products Co. Gardena, CA) and stored in a plastic Presto® bag (26.8 X 27.9cm, Presto Products Company, Appleton, WI), and labeled with the date, bromeliad species, and location. In the laboratory, larvae were identified to species and recorded. To determine adult sex ratios, a small portion (about 1/5) of larvae from samples at each site was transferred to separate mosquito breeders (BioQuip Products Co.) for rearing to adults. Upon emergence, adults were identified and sex was determined.

To investigate the occurrences of either a single or mixed mosquito species assemblage in the axils, all axils with mosquito larvae were placed into1 of 7 categories: *Wy. mitchellii only, Ae. albopictus only, Cu. quinquefasciatus* only, *Wy. mitchellii and Ae. albopictus (Wy. & Ae), Wy. mitchellii and Cu. quinquefasciatus (Wy. & Cu.), Ae. albopictus and Cu.*

quinquefasciatus (Ae. & Cu.), and *Wy. mitchellii, Ae. albopictus and Cu. quinquefasciatus (Wy. & Ae. & Cu.)*. In this study, *V. aff. regina* from four different sites were selected. Percentages were calculated from the axils with mosquito larvae within each category at each survey site. The t-test was used to test the significance of the difference between the numbers of mosquito larvae in the central cups and those in the leaf axils of *V. aff. regina*. The data were transformed by the square root method, and then tested for equality of variances before analysis (Analytical Software 1996). Chi-square tests with correction for continuity were employed to test the null hypotheses that the sex ratio was not different from 1:1 (Snedecor and Cocharan 1967).

Results

Four species of mosquitoes, including three noxious species, *Wy. mitchellii, Ae. albopictus*, and *Cu. quinquefascatus*, and one beneficial species, *T. amboinensis*, were recovered in samples. Because the population density of *T. amboinensis* was very low (less than one larva per 100 axils and only found in two sites), we focus mainly on the three noxious species (Table 1).

Abundance affected by bromeliad species. The mean capacities of the central axils of *Neoregelia sp*. and *N*. '*Macwilliamsii*' were 67 ml and 24 ml, respectively. For *V. aff. regina*, the mean capacities of central axils and leaf axils were 691 ml and 740 ml, respectively, which were not significant different (t test, t=0.6, df=18, p=0.554).

Wyeomyia mitchellii and *Ae. albopictus* larvae were recovered from all three species of bromeliads while *Cu. quinquefasciatus* was found only in *V. aff. regina*. Per axil, more *Wy. mitchellii* and *Ae. albopictus* larvae were recovered from *V. aff. regina* than from *Neoregelia sp*. or *N*. '*Macwilliamsii*' at all sites (Table 1). Furthermore, on average, *V. aff. regina* had 39 leaf axils, of which 30 held water, and *V. aff. regina* produced more mosquitoes (including all three species) than the other two bromeliad species, in which the central axils only held water consistently. More *Ae. albopictus* were collected from *Neoregelia sp*. than from *N*. '*Macwilliamsii*'. For example, at the National Tropical Botanical Garden the mean *Ae. albopictus* larvae per axil was 0.25 from *Neoregelia sp*. and 0.04 from *N*. '*Macwilliamsii* (Table 1). There was a six-fold difference in larval numbers between these two bromeliad species; however, at the same location, more *Wy*. m*itchellii* were collected from *N*. '*Macwilliamsii*' than from *Neoregelia sp*. (almost two-fold difference).

Abundance affected by site. Location seemed to play an important role in determining the abundance of mosquito larvae in bromeliads (Table 1). *Culex quinquefasciatus* was the most abundant species sampled from *V. aff. regina* at the Plantation Garden, but none was found at Kilauea nursery. *Wyeomyia mitchellii* was the most abundant species from *V. aff. regina* at the National Tropical Botanical Garden, but was the least abundant at the Plantation Garden. Furthermore, for *Ae. albopictus*, there was approximately a 15-fold difference between numbers of larvae in the axils of *V. aff. regina* from the Plantation Garden and those from the same plant at Kilauea nursery. Also, there was a five-fold difference in numbers of *Ae. albopictus* larvae in the axils of *Neoregelia* sp. between these two sites (Table 1).

Abundance affected by type of axils. Although the central axils and leaf axils of *V. aff. regina* held similar amounts of water, more *Cu. quinquefasciatus* larvae were recovered from leaf axils (t=2.31, df=188, p=0.022) (Table 2). There were no significant differences between types of axils either for the combined number of mosquito larvae of the three species (t=1.88, df=188, p=0.061), or *Wy. mitchellii* and *Ae. albopictus* species alone (t=0.92, df=188, p=0.359; t=1.55, df=188, p=0.123).

Occurrence of single and mixed species. All three species of mosquitoes bred alone, or in combination of two or three species in axils of *V. aff. regina*. But, there was not a clear

Table 1. Mosquito larvae recovered from samples collected from three species of bromeliad within the survey sites (mean±SE).

Survey site	Plant species	No axils	*Wyeomyia mitchellii*	*Aedes albopictus*	*Culex quinquefasciatus*
Plantation garden	*V. aff. Regina*	70	0.39 ± 0.22	6.11 ± 1.24	23.13 ± 4.14
	Neoregelia sp.	136	0.01 ± 0.01	0.94 ± 0.22	0
	N. 'Macwilliamsii'	40	0	0.75 ± 0.22	
National Tropical Botanical Garden	*V. aff. Regina*	54	8.7 ± 3.14	1.69 ± 0.46	6.35 ± 1.31
	Neoregelia sp.	100	3.41 ± 0.53	0.25 ± 0.08	0
	N. 'Macwilliamsii'	184	6.46 ± 0.47	0.04 ± 0.02	0
Kilauea Nursery	*V. aff. Regina*	38	5.3 ± 0.5	0.4 ± 0.2	0
	Neoregelia sp.	73	1.19 ± 0.3	0.18 ± 0.1	0
Princeville Nursery	*V. aff. Regina*	28	12.9 ± 3.42	4.68 ± 1.19	17.9 ± 6.05
	N. 'Macwilliamsii'	75	3.09 ± 0.42	0	0

Table 2. Mosquito larvae recovered from central axils and leaf axils of *Vriesea aff. Regina* (mean±SE).*

	No. axils	*Wy. mitchillii*	*Ae. albopictus*	*Cu. quinquefasciatus*	All three species sampled
Central axils	53	5.94 ± 0.82a	2.91 ±0.4a	9.17 ±1.26a	18.0 ±2.48a
Leaf axils	137	4.50 ± 0.38a	3.64 ± 0.31a	17.58 ±1.5b	25.72 ±2.19a

*Means within columns followed by the same letter are not statistically different at $P \leq 0.05$.

Table 3. Occurrence (% of samples) of three species of mosquito larvae in leaf axils of *Vriesea aff. Regina* from four sites.

Sites	No. samples	*Cu. quinquefasciatus* only	*Ae. albopictus* only	*Wy. mitchellii* only	*Cu. + Ae.*	*Ae. + Wy.*	*Cu. + Wy.*	*Cu. + Ae. + Wy.*
Plantation garden	70	24	10	4	57	1	0	2.8
National Tropical Botanical Garden	53	11	9.4	41.5	11.3	22.6	1.9	1.8
Kilauea Nursery	36	0	0	88.9	0	11.1	0	0
Princeville Nursery	25	12	0	0	8	48	4	28

occurrence pattern (Table 3). For both single species and mixed species, they were higher at one site, but were lower at another site. For example, at the Plantation Garden, the occurrence of *Cu. quinquefasciatus* alone is 24%, but at the Kilauea nursery, its occurrence is 0. At the Princeville nursery, the occurrence of *Wy. mitchellii* and *Ae. albopictus* was 48%, but at the Plantation Garden, the occurrence was 1%.

Sex ratio. Larvae collected from bromeliads at the 4 locations were reared out to adults as follows: 172 females and 146 males of *Wy.* m*itchellii*, 143 female and 107 males of *Ae. albopictus,* and 487 females and 594 males of *Cu. quinquefasciatus.* The sex ratios of *Wy.* m*itchellii,* and *Cu. quinquefasciatus* were consistent with the 1:1 sex ratio hypothesis (X^2 =1.97 and 3.59, df=1, respectively; $p>0.05$); however, the sex ratio of *Ae. albopictus* was skewed towards females (X^2=4.90; df=1, $p<0.05$).

Discussion

Our results agree with earlier study (Frank et al, 1977) that larger plants contain more mosquitoes. But, our data also show that in certain range of size, it is possible that more *Wy. mitchellii* breed in smaller size plants perhaps due to its preference on plant species.

Frank et al. (1977) found that two *Wyeomyia spp*. present in the leaf axils of a bromeliad varied seasonally and from one locality to another. Our results show that the abundance of other three mosquito species also is affected by location. How the site factor affects mosquito populations is not clear. But some biotic and abiotic factors such as the vegetation and microclimate at the different sites may affect population establishment and abundance.

More organic debris such as flowers and seeds were found in the leaf axils than in the central axils of *V. aff. regina*. As a result, more *Cu. quinquefasciatus* recovered from leaf axils than central axils, indicating its preference for the water with more organic materials (Service, 1996). Frank et al. (1976) found that oviposition of adult *Wy. vanduzeei* was not affected by the presence of organic infusion, or infusion plus organic debris in the leaf axils. It appears that both *Wy. mitchellii* and *Ae. albopictus* in this study have similar responses to the water quality.

When different species with identical niches occur at the same place and time, severe interspecific competition can occur (Price 1975). This is likely the case with the three species of mosquito larvae in bromeliad axils in the present study. Also, certain factors may affect the competition of these three species. For example, more organic materials in water will favor the *Cu. quinquefasciatus* larvae and may affect the other two species negatively (Service 1996). No *Cu. quinquefasciatus* larvae were found from smaller species of bromeliad (Table 1) indicating the volume of water in leaf axils is an important factor in affecting adult oviposition.

Our survey did not provide enough data on competition among the three mosquito species in bromeliads. However, the survey provided enough information on their coexistence. The competition and coexistence of bromeliad breeding mosquitoes will provide sources to better understand the community structure and their population dynamics.

The beneficial species *T. amboinensis* plays a very minor role in controlling mosquitoes in bromeliads due to its very low population density. If it has highly domesticated behavior as Nakagawa (1963) indicated, it should have higher population density in bromeliads, which are common yard plants in Hawaii. Further studies are necessary to determine the major factors influencing the abundance of *T. amboinensis.*

Overall, our results indicate that bromeliads are an important resource for mosquitoes in Hawaii, producing large numbers of *Wy.* m*itchellii, Ae. albopictus* and *Cu. quinquefasciatus*. It is apparent that more attention should be given to these plants since they are very common in residential areas and thus may enhance mosquito-human contact there.

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